ภาคผนวก ก
อาหารเลี้ยงเชื้อและสารละลาย

1.1. LB Medium (Luria-Bacterial Medium)

Per litre:

To 950 ml of deionised H_2O add:

bacto-tryptone 10 g

bacto-yeast extract 5 g

NaCl 10 g

Shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (0.2 ml). Adjust the volume of the solution to 1 litre with deionised H_2O. Sterilise by autoclaving for 20 min at 15lb/sq. in. on liquid cycle.

1.2. SDS-PAGE

- Solutions for preparing 12% resolving SDS-polyacrylamide gel

Solution component	**Component volume (ml)**		
	5 ml	**10 ml**	**20 ml**
H_2O	1.6	3.3	6.6
30% (w/v) acrylamide mix	2.0	4.0	8.0
1.5 M Tris (pH 8.8)	1.25	2.5	5.0
10% SDS	0.05	0.1	0.2
10% ammonium persulfate (freshly prepared)	0.05	0.1	0.2
TEMED	2 µl	4 µl	6 µl

- Solutions for preparing 5% stacking SDS-polyacrylamide gel

Solution component	**Component volume (ml)**	
	2 ml	**5 ml**
H_2O	1.4	3.4
30% (w/v) acrylamide mix	0.33	0.83
1.0 M Tris (pH 6.8)	0.25	0.63

10% SDS	0.02	0.05
10% ammonium persulfate (freshly prepared)	0.02	0.05
TEMED	2 µl	5 µl

- **Buffers for SDS-PAGE**

SDS-gel loading buffer (3 x stock)

150 mM Tris.Cl (pH6.8)

300 mM dithiothreitol

6% SDS (electrophoresis grade)

0.3 % bromophenol blue

30% glycerol

- **Tris-Glycine electrophoresis buffer (5 x stock)**

250 mM Tris.Cl (pH 8.3)

1.25 M glycine (electrophoresis grade) (pH 8.3)

0.5 % SDS

- **Staining solution with Coomassie Brilliant Blue for Protein**

Dissolve 0.25 g of Coomassie Brilliant Blue R250 in 90 ml of methnol:H_2O (1:1v/v) and 10 ml of glacial acetic acid. Filter the solution through a Whatman No. 1 filter to remove any particulate matter.

- **Destaining Solution for Coommassie Stain**

30% methanol

10% acetic acid

dH_2O is added to bring volume to 100 ml.

1.3. Preparation of competent cells

1. Streak *E. coli* host cells on an LB plate+100 μg/ml Amp)
2. Allow cells to grow at 37°C overnight
3. Place one colony in 10 mL LB media (+antibiotic selection if necessary), grow overnight at 37°C
4. Transfer 5 mL overnight DH5a culture into 500 mL LB media in 2-L flask
5. Allow cell to grow at 37°C (250 rpm), until OD_{600}= 0.6 (~2-3 hours)
6. Transfer cells to 2 centrifuge bottles (250 mL), and place cells on ice for 20 mins
7. Centrifuge cells in Sorval GSA rotor at 4°C for 10 mins at 3,000 g (2500 rpm). **Cells must remain cold for the rest of the procedure**
8. Pour off media and resuspend cells in 30 mL of **cold** 0.1 M $CaCl_2$. Transfer the suspended cells into 50 mL polypropylene falcon tubes, and incubate on ice for 30 mins
9. Centrifuge cells using rotor at 4 °C for 10 mins at 3,000 g
10. Pour supernatant and re-suspend cells (by pipetting) in 8 mL cold 0.1M $CaCl_2$ containing 15% glycerol. Transfer 100 μL into (1.5 mL) Eppendorff tubes placed on ice. Freeze the cells in liquid nitrogen. Cells stored at -80°C can be used for transformation for up to ~6 months.

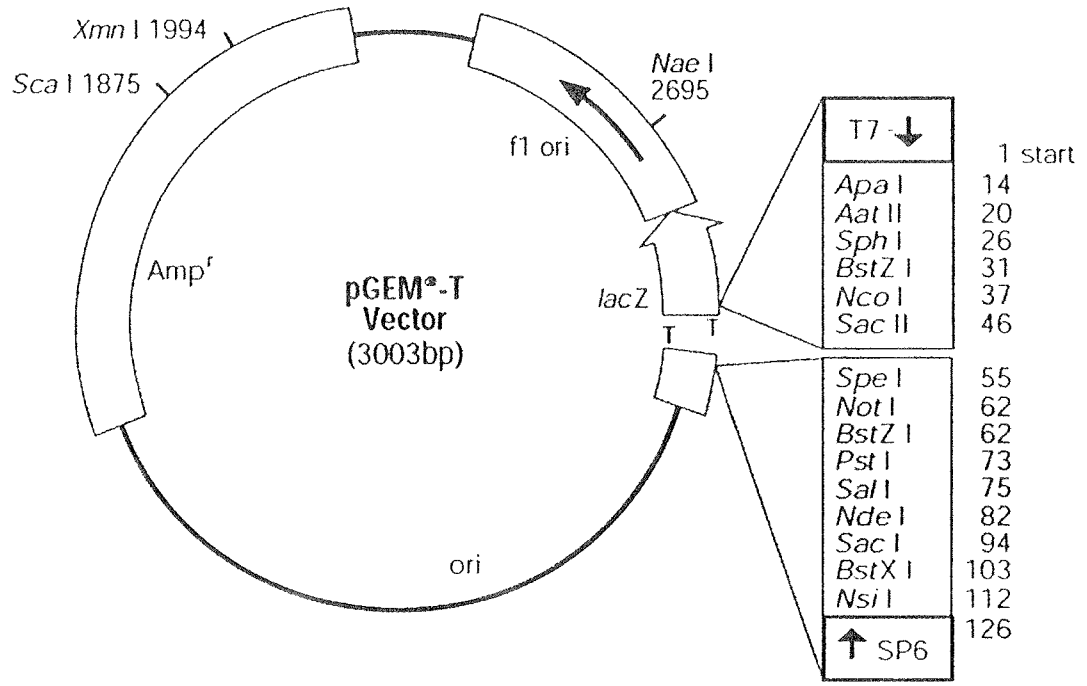
1.4. Buffers for Western blot analysis

Phosphate-buffered saline plus Tween 20 (PBS-T)

Dissolve 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.2 g of KH_2PO_4 in 800 ml of distilled H_2O. Adjust the pH to 7.4 with HCl. Add H_2O to 1 litre. 1% (v/v) of Tween 20 is added and stirred to prior to use.

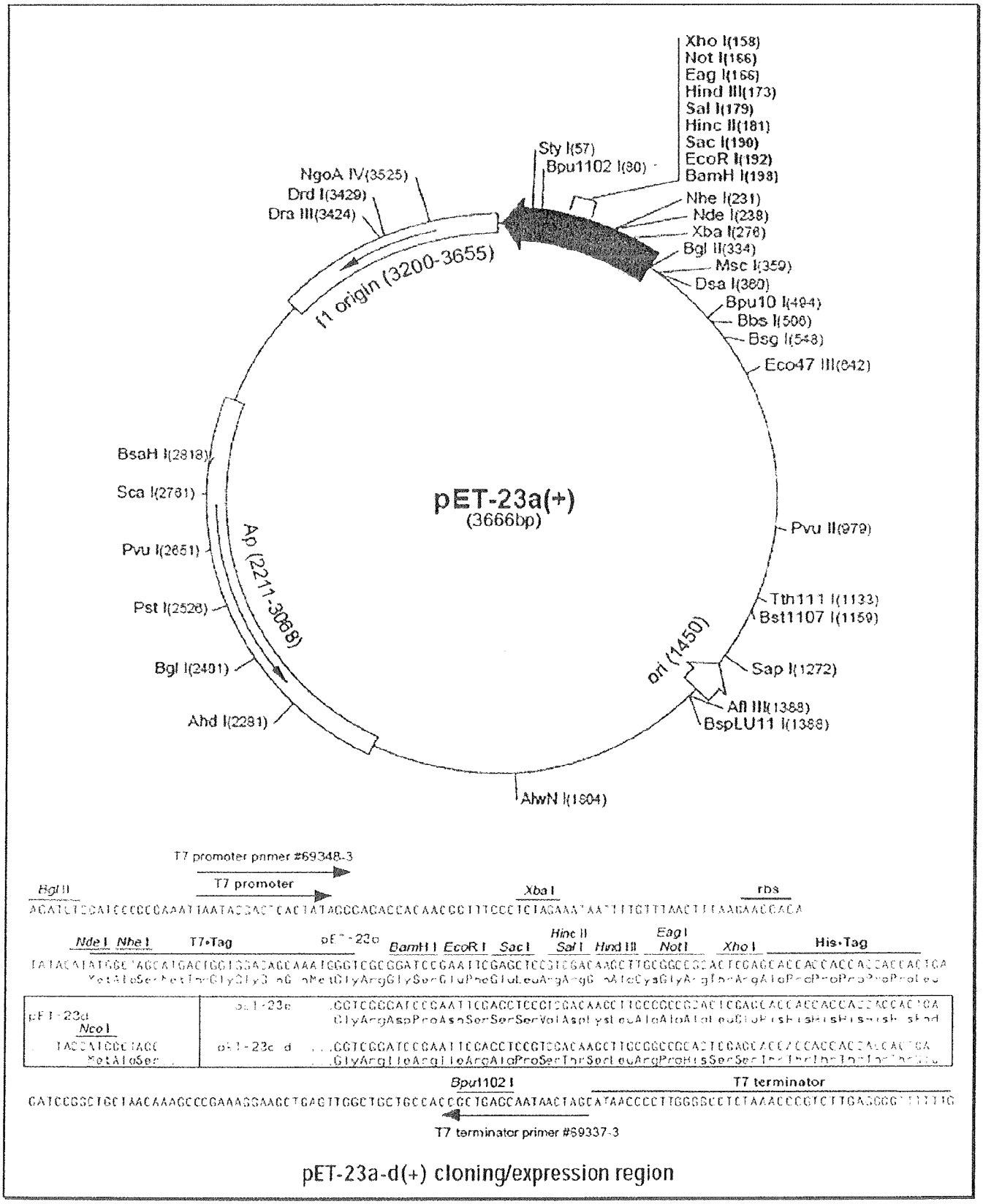
ภาคผนวก ข

- แผนที่พลาสมิด pGEM®-T cloning vector



pGEM®-T Vector Sequence reference points:	
T7 RNA Polymerase transcription initiation site	1
SP6 RNA Polymerase transcription initiation site	126
T7 RNA Polymerase promoter	2987-6
SP6 RNA Polymerase promoter	121-143
multiple cloning site	10-113
*lac*Z start codon	165
lac operon sequences	2824-2984, 151-380
lac operator	185-201
β-lactamase coding region	1322-2182
phage f1 region	2368-2823
binding site of pUC/M13 Forward Sequencing Primer	2944-2960
binding site of pUC/M13 Reverse Sequencing Primer	161-177

- แผนที่พลาสมิด pET23a-d(+) expression vector



pET-23a-d(+) cloning/expression region

ภาคผนวก ข

การนำเสนอผลงาน

1. ผลงานตีพิมพ์ในวารสารนานาชาติ 2 ผลงาน

- Siritapetawee J, Prinz H, Krittanai C & **Suginta W** (2004) Expression, refolding of Omp38 from *Burkholderia pseudomallei* and *B. thailandensis*, and its function as a diffusion porin. *Biochem. J.* 384, 609–617. (JIF2008 = 4.317)
- Siritapetawee J, Prinz H, Samosornsuk W, Ashley RH & **Suginta W** (2004) Functional reconstitution, gene isolation and topology modelling of porins from *Burkholderia pseudomallei* and *B. thailandensis*. *Biochem. J.* 377, 579-587. (JIF2008 = 4.317)

2. ผลงานนำเสนอในรูปบรรยายหรือโปสเตอร์ในที่ประชุมระดับนานาชาติหรือระดับชาติ 4 ผลงาน

- **Suginta W.** On the structure and function of bacterial porins and chitinases. A Departmental Special Seminar, Department of Life Sciences, Faculty of Sciences and Agriculture, The University of the West Indies, Trinidad and Tobago, May 15th, 2006. *Invited oral presentation.*
- Siritapetawee J, Prinz H, Krittanai C, Ashley RH & **Suginta W** Porin from *Burkholderia pseudomallei* and B. *thailandensis*. In 30th FEBS Congress & 9th IUBMB Meeting, Budapest, Hungary, July2nd-7th, 2005. *FEBS J. Vol.* 272, *Supp.* 1, P389. *Poster presentation.*
- Siritapetawee J & **Suginta W** Expression and refolding of Omp38 from *Burkholderia pseudomallei* and *Burkholderia thailandensis*, and their function as a non-specific channel. The 15th Annual Meeting of the Thai Society for Biotechnology. February, 3rd-6th, 2004. P5. *Poster presentation.*
- Siritapetawee J, Ashley RH, Prinz H, Samosornsuk W & **Suginta W** Identification of *Burkholderia* porins using MALDI-TOF and nanoelctrospray MS. 1st Protein Symposium Network, Mahidol, Thailand, August 29th-30th, 2002. *Invited oral Presentation.*